FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: 3276.1000000

U S APPLICATION NO. (If known, see 37 CFR 1 5): 09/937649

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/02910	31 March 2000 (31.03.00)	01 April 1999 (01.04.99)

TITLE OF INVENTION

MONOCLONAL ANTIBODIES AGAINST HUMAN PROTEIN MCM3, PROCESS FOR THEIR PRODUCTION, AND THEIR USE

APPLICANT(S) FOR DO/EO/US Johannes Gerdes, Thomas Scholzen, Elmar Endl, Claudia Wohlenberg, Bettina Baron-Luhr, Margrit Hahn, Patricia Prilla, Johanna Szuwinski and Rolf Knippers

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [ ] Other items or information:

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/937649	PCT/EP00/02910	3276.1000000

21. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		CALCULATIONS PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	24 - 20 =	4	x $18.00	$ 72.00
Independent claims	4 - 3 =	1	x $80.00	$ 80.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1012.00
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$
SUBTOTAL =				$ 1012.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 1012.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$
TOTAL FEES ENCLOSED =				$ 1012.00
			Amount to be refunded:	$
			charged:	$

a. [X] A check in the amount of $ 1012.00 to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-0380 . A duplicate copy of this sheet is enclosed.

d. [ ] Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

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22,592
REGISTRATION NUMBER

IN THE UNITED STATES RECEIVING OFFICE (RO/US)
Designated/Elected Office (DO/EO/US)

U.S. National Phase of International Application No.:	PCT/EP00/02910
International Filing Date:	31 March 2000
Earliest Priority Date:	01 April 1999
Applicants:	Johannes Gerdes, Thomas Scholzen, Elmar Endl, Claudia Wohlenberg, Bettina Baron-Luhr, Margrit Hahn, Patricia Prilla, Johanna Szuwinski and Rolf Knippers
Title:	MONOCLONAL ANTIBODIES AGAINST HUMAN PROTEIN MCM3, PROCESS FOR THEIR PRODUCTION, AND THEIR USE
Attorney's Docket No.:	3276.1000-000

Date: 27 September 2001
EXPRESS MAIL LABEL NO. EL762235141US

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows:

In the Specification

At page 1, following the title, please insert the following paragraph:

--RELATED APPLICATIONS

This application is the U.S. National Phase of International Application No. PCT/EP00/02910, filed March 31, 2000, designating the United States and claiming priority under 35 U.S.C. § 119 to German Application No. DE 199 15 057.5, filed April 1, 1999.--

In the Claims

Cancel Claims 1-24.

Add new Claims 25 - 48, as follows:

25. A monoclonal antibody specific for human Mcm3.

26. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically, whereby the monoclonal antibody has the same properties as the monoclonal antibody of the hybridoma cell line with the deposit number DSM ACC2388.

27. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically, whereby the epitope of the monoclonal antibody of the hybridoma cell line with the deposit number DSM ACC2388 is detected.

28. The monoclonal antibody according to Claim 25 altered biochemically, by molecular biology or synthetically.

29. The monoclonal antibody according to Claim 25, which is produced by the hybridoma cell line with the deposit number DSM ACC2388.

30. A hybridoma cell line which expresses a monoclonal antibody specific for human Mcm3.

31. A hybridoma cell line according to Claim 30, whereby the hybridoma cell line is the cell line with the deposit number DSM ACC2388.

32. A method of detecting human Mcm3 using the monoclonal antibody of Claim 25.

33. A method for the immunohistological, immunocytological or immunobiochemical detection of human Mcm3 in a sample using the monoclonal antibody of Claim 25.

34. A method of Claim 33 wherein the sample is selected from serum, sputum, urine, or liquor.

35. A method of Claim 33 wherein the sample is tissue or a fine needle aspiration product.

36. A method of Claim 33 wherein the method is an immunobiochemical method selected from ELISA, RIA, Western Blot, Far Western Blot, immunoprecipitation and affinity chromatographic steps.

37. A method of Claim 33 wherein the method is an immunocytological method selected from FACS and MACS.

38. A method of Claim 33 wherein the method is an immunohistological method selected from fluorescence, radioactive, enzymatic and chemiluminescence methods.

39. A process for the production of the antibody according to Claim 25, characterized in that an animal is immunized with human Mcm3, and monoclonal antibodies are

obtained after fusion of spleen cells of the animal with myeloma cells which comprises the steps:

(i) initial screening of the hybridoma by means of an immunobiochemical method;

(ii) screening of the hybridoma that were positive in step (i) by means of an immunohistochemical method.

40. A process for the production of purified human Mcm3 employing the monoclonal antibody according to Claim 25.

41. A process for the production of purified human Mcm3, characterized in that the process comprises an affinity chromatography step with a monoclonal antibody according to Claim 25.

42. A process for the production of purified human Mcm3 comprising an immunoprecipitation step with a monoclonal antibody according to Claim 25.

43. A diagnostic composition comprising a monoclonal antibody according to Claim 25.

44. A method for the production of a preparation for the therapy of tumors, allergies, autoimmunopathies, scar formation, inflammation and rheumatic diseases as well as the suppression of defense reactions of transplantations employing the monoclonal antibody of Claim 25.

45. A pharmaceutical composition comprising a monoclonal antibody of Claim 25 together with a pharmaceutical acceptable adjuvant.

46. A diagnostic kit comprising the monoclonal antibody according to Claim 25.

47. A diagnostic kit according to Claim 46 for the combined detection of the expression of Mcm3, Ki-67 and p27 for tumor diagnosis.

48. A method of preventing or treating a disease caused by or contributed by the activity or level of Mcm3 expression, comprising administering to the subject an effective amount of a pharmaceutical composition comprising an antibody according to Claim 25 together with a pharmaceutically acceptable carrier.

REMARKS

This application is the United States national application arising from International Application No. PCT/EP00/02910.

The original claims of the International Application have been canceled and substituted with new claims of the same scope but in a better format for United States applications.

For the convenience of the Examiner, a complete set of the new claims is attached to this Amendment under the heading "Claims Pending, as Amended Herein".

In the International Preliminary Examination Report issued in the International Application on May 28, 2001, all claims having the scope of those now present in the application were indicated to be novel, nonobvious and useful.

Applicant's Attorney hereby authorizes the Patent Office to charge any additional fees to Deposit Account No. 08-0380. A copy of this letter is enclosed for accounting purposes.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

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Dated: 9/27/01

U.S. National Phase of PCT/EP00/02910

CLAIMS PENDING, AS AMENDED HEREIN

25. A monoclonal antibody specific for human Mcm3.

26. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically, whereby the monoclonal antibody has the same properties as the monoclonal antibody of the hybridoma cell line with the deposit number DSM ACC2388.

27. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically, whereby the epitope of the monoclonal antibody of the hybridoma cell line with the deposit number DSM ACC2388 is detected.

28. The monoclonal antibody according to Claim 25 altered biochemically, by molecular biology or synthetically.

29. The monoclonal antibody according to Claim 25, which is produced by the hybridoma cell line with the deposit number DSM ACC2388.

30. A hybridoma cell line which expresses a monoclonal antibody specific for human Mcm3.

31. A hybridoma cell line according to Claim 30, whereby the hybridoma cell line is the cell line with the deposit number DSM ACC2388.

32. A method of detecting human Mcm3 using the monoclonal antibody of Claim 25.

33. A method for the immunohistological, immunocytological or immunobiochemical detection of human Mcm3 in a sample using the monoclonal antibody of Claim 25.

34. A method of Claim 33 wherein the sample is selected from serum, sputum, urine, or liquor.

35. A method of Claim 33 wherein the sample is tissue or a fine needle aspiration product.

36. A method of Claim 33 wherein the method is an immunobiochemical method selected from ELISA, RIA, Western Blot, Far Western Blot, immunoprecipitation and affinity chromatographic steps.

37. A method of Claim 33 wherein the method is an immunocytological method selected from FACS and MACS.

38. A method of Claim 33 wherein the method is an immunohistological method selected from fluorescence, radioactive, enzymatic and chemiluminescence methods.

39. A process for the production of the antibody according to Claim 25, characterized in that an animal is immunized with human Mcm3, and monoclonal antibodies are obtained after fusion of spleen cells of the animal with myeloma cells which comprises the steps:

(i) initial screening of the hybridoma by means of an immunobiochemical method;

(ii) screening of the hybridoma that were positive in step (i) by means of an immunohistochemical method.

40. A process for the production of purified human Mcm3 employing the monoclonal antibody according to Claim 25.

41. A process for the production of purified human Mcm3, characterized in that the process comprises an affinity chromatography step with a monoclonal antibody according to Claim 25.

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43. A diagnostic composition comprising a monoclonal antibody according to Claim 25.

44. A method for the production of a preparation for the therapy of tumors, allergies, autoimmunopathies, scar formation, inflammation and rheumatic diseases as well as the suppression of defense reactions of transplantations employing the monoclonal antibody of Claim 25.

45. A pharmaceutical composition comprising a monoclonal antibody of Claim 25 together with a pharmaceutical acceptable adjuvant.

46. A diagnostic kit comprising the monoclonal antibody according to Claim 25.

47. A diagnostic kit according to Claim 46 for the combined detection of the expression of Mcm3, Ki-67 and p27 for tumor diagnosis.

48. A method of preventing or treating a disease caused by or contributed by the activity or level of Mcm3 expression, comprising administering to the subject an effective amount of a pharmaceutical composition comprising an antibody according to Claim 25 together with a pharmaceutically acceptable carrier.

Monoclonal antibodies against human protein Mcm3, process for their production, and their use.

DESCRIPTION

Technical field

The present invention relates to monoclonal antibodies against human protein Mcm3, processes for their production and their use.

Prior art

Mcm proteins were first described in the barm S. cerevisiae. It is known, that these proteins play an important role in the initiation of DNA replication, which was shown in the barm by its decisive role in the transmittance of extra chromosome DNA segments, minichromosomes (Maine et al., Genetics, 1984, 106:365-385). This feature was the basis for the naming for these proteins, minichromosome maintenance, Mcm. The proteins of the Mcm family are highly conserved with respect to evolution.

At present six proteins (Mcm2, Mcm3, Mcm4, Mcm5, Mcm6, Mcm7) are described in the human system which with other cell cycle dependent structures form a protein complex that is necessary for DNA replication, and which were already postulated as DNA replication licence factors by J.J. Blow and R.A. Laskey in 1988 (Nature, 332:546-548). Mcm3 protein plays an important role by forming a biochemical strong bond with Mcm5 (A. Richter, R. Knippers, Eur. J. Biochem., 1997, 247:136-141). Since Mcm3 and the other members of the Mcm family have such a basic function in the cell cycle, detection systems are desired, preferably immunobiochemical and

immunohistological detections. Such detections are of need because new parameters for medical diagnosis, preferably in cancer diagnosis, can be achieved therewith.

It is known that human Mcm protein is immunogenic in the rabbit (Thommes et al., Nucleic Acid Res., 1992, 20:1069-1074). But the known polyclonal antisera either do not react monospecifically in immunobiochemical analyses (Western Blot) and/or are not applicable quickly and without problems in routine immunohistology (Hu, B., et al., Nucleic Acid Res., 1993, 21: 5289-5293). Therefore, there is no tool at hand that can serve as a detection method for Mcm3 in medical diagnosis.

Summary of the invention

The object of the present invention is therefore to provide means that detect quickly and monospecifically Mcm3 protein in biochemical and also in histological systems conducted alone or together in combination. This detection can be conducted alone or in combination with other known markers.

According to the invention this is achieved by a monoclonal antibody directed against Mcm3 protein and being applicable both in immunobiochemical and in immunohistochemical detection systems, whereby these detections can be conducted alone or in combination.

Further, hybridomas producing monoclonal antibodies according to the invention are disclosed.

Another aspect of the invention is the provision of diagnostic compositions and detection kits comprising the monoclonal antibody according to the present invention.

Yet another aspect is the use of the monoclonal antibody according to the present invention for the detection of Mcm3 in a sample.

Moreover, processes are disclosed relating to the production of a monoclonal antibody and hybridoma, respectively, according to the present invention.

Finally, the present invention relates to pharmaceutical preparations and medicines containing the monoclonal antibody and the use of the monoclonal antibody for the preparation of a medicament for the treatment of certain diseases.

Also, within the scope of the invention are methods for treating diseases or disorders which are associated with an aberrant McM3 level or activity or which can benefit from modulation of the activity or level of McM3. The methods comprise administering, e.g., either locally or systemically to a subject, a pharmaceutically effective amount of a composition comprising an MCm3 antibody according to the present invention.

Short description of the figures

Fig.1 shows a Western Blot using a monoclonal antibody according to the present invention (right side) and a polyclonal antibody known in the art (left side). It is clearly demonstrated that the antibody according to the present invention recognises only one band while the polyclonal antibody detects further bands in the range of 90 to 50 kDa.H denotes HeLa cells and C denotes CHO cells.

Detailed description of the invention

According to the invention a monoclonal antibody directed against Mcm3 protein and being applicable both in immunobiochemical and in immunohistochemical detection systems, whereby these detections can be conducted alone or in combination is provided.

The monoclonal antibody according to the present invention can be obtained from any animal or the human being, whereby the monoclonal antibodies of the mouse are preferred.

Further, the monoclonal antibody may be altered biochemically, by genetic manipulation, or it may be synthetic, with the antibody possibly lacking portions completely or in parts, said portions being necessary for the recognition of Mcm3 and being substituted by others imparting further advantageous properties to the antibody.

A hybridoma cell line producing a preferred monoclonal antibody of the present invention, namely, a monoclonal mouse antibody with said above-mentioned detection, was deposited at the Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig under the number DSM ACC2388 on February 16, 1999.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) Mcm3. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme

such as pepsin. The invention provides monoclonal antibodies that bind Mcm3. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of Mcm3. A monoclonal antibody composition thus typically displays a single binding affinity for Mcm3 with which it immunoreacts.

A disease, a disorder or condition "associated with" or "characterized by" an aberrant McM3 activity refers to a disease, disorder or condition in a subject which is caused by or contributed to by an aberrant MCm3 activity.

The term "treating" as used herein is intended to encompass curing as well as ameliorating at least one symptom of the condition or disease.

Monoclonal anti-Mcm3 antibodies can be prepared by immunizing a suitable subject with an Mcm3 immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed Mcm3 protein or a chemically synthesized Mcm3 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agents. Immunization of a suitable subject with an immunogenic Mcm3 preparation induces an anti-Mcm3 antibody response.

The anti-Mcm3 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized Mcm3. If desired, the antibody molecules directed against Mcm3 can be isolated from the mammal (e.g., from the blood) and further purified by well known

techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Koehler and Milstein (1975) Nature 256:495-497) (see also, Brown et al. (1981) J. Immunol. 127:539-46; Brown et al. (1980) J. Biol. Chem. 255:4980-83; Yeh et al. (1976) Proc. Natl. Acad. Sci. US.4 76:2997-3 1; and Yeh et al. (1982) Int. J. Cancer 29:269-75), the more recent human B cell hybridoma technique (Kozbor et al. (1983) Immunol Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in Monoclonal Antibodies: A new Dimension in Biological Analyses, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) Yale J: Biol. Med., 54:387402; M. L. Gefter et al. (1977) Somatic Cell Genet. 3:23136). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with Mcm3 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds Mcm3.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating anti-Mcm3 monoclonal antibodies (see, e.g., G. Galfre et al. (1977) Nature 266:55052; Gefter et al. Somatic Cell Genet., cited supra; Lerner, Yale J. Biol. Med, cited supra; Kenneth, Monoclonal Antibodies, cited supra). Moreover, the

ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS 1/1-Ag4- 1; P3x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind Mcm3, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-Mcm3 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with Mcm3 to thereby isolate immunoglobulin library members that bind Mcm3. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP® Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents

particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. PCT International Publication No. WO 92/18619; Dower et al. PCT International Publication No. WO 91/17271; Winter et al. PCT International Publication WO 92/20791; Markland et al. PCT International Publication No. WO 92/15679; Breitling et al. PCT International Publication WO 93/01288; McCafferty et al. PCT International Publication No. WO 92/01047; Garrard et al. PCT Intemational Publication No. WO 92/09690; Ladner et al. PCT International Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370- 1372; Hay et al. (1992) Hum. Antibod. Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J 12:725-734; Hawkins et al. (1992) J. Mol. Biol. 226:889-896; Clarkson et al. (1991) Nature 352:624-628; Gram et al. (1992) Proc. Natl. Acad. Sci. USA 89:3576-3580; Garrad et al. (1991) Bio/Technology 9:1373-1377; Hoogenboom et al. (1991) J\tUC. Acid Res. 19:4133-4137; Barbas et al. (1991) Proc. Natl. Acad. Sci. USA 88:7978-7982; and McCafferty et al. Nature (1990) 348:552-554.

Additionally, recombinant anti-Mcm3 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson et al. International Application No. PCT/US86/02269; Akira, et al. European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison et al. European Patent

Application 173,494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Patent No. 4,816,S67; Cabilly et al. European Patent Application 12 S,023; Better et al. (1988) Science 240:1041-1043; Liu et al. (1987) Proc. Natl Acad. Sci. USA 84:3439-3443; Liu et al. (1987) J. Immunol. 139:3521-3526; Sun et al. (1987) Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al. (1987) Canc. Res. 47:999-1005; Wood et al. (1985) Nature 314:446-449; and Shaw et al. (1988) J. Natl. Cancer Inst. 80: 1553-1559); Morrison, S. L. (1985) Science 229: 1202- 1207; Oi et al. (1986) Bio Techniques 4:214; Winter U.S. Patent 5,225,539; Jones et al. (1986) Nature 321:552-525; Verhoeyan et al. (1988) Science 239: 1534; and Seidler et al. (1988) J. Immunol. 141:4053-4060. An anti-Mcm3 monoclonal antibody can be used to isolate Mcm3 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-Mcm3 antibody can be used to detect Mcm3 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of Mcm3. Anti-Mcm3 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, (-galactosidase, or acetylcholinesterase); examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine

fluorescein, dansyl chloride, Cy-dyes, Alexa-dyes or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ; ^{35}S or ^{3}H.

Preferably, monoclonal antibodies according to the invention can be produced with the initial screening strategy described further below. Since a plurality of prepared hybridoma are either not monospecifically against Mcm3 protein or are applicable only in immunobiochemical detection systems but not in immunohistological systems and vice versa, initial examination of generated hybridoma cells requires this strategy to produce monoclonal antibodies according to the invention which have both properties.

For the production of genetically altered and/or synthetic antibodies having the properties according to the invention one can start e.g. from monoclonal antibodies obtained as described above. For this it is suitable to analyse the Mcm3 binding regions of the monoclonal antibodies and to identify the parts that are necessary and unnecessary for the detection described above. Then the necessary portions can be modified and the unnecessary portions can be eliminated completely or in part and can be substituted, respectively, by portions imparting further advantageous properties to the antibodies. Also, portions not within the binding regions of the antibodies can be modified, eliminated, or substituted. It is known by the skilled person that particularly the DNA recombination technology is suitable for the above measures.

Monoclonal antibodies according to the invention are distinguished by detecting Mcm3 monospecifically both in biochemical and histological detecting systems. The antibodies are therefore suitable for the fast detection of a Mcm3 expression in very different samples.

Within the present context, the term ``sample'' is intended to cover all types of samples suitable for the purpose of the invention. Examples of such samples are serum, sputum, urine, liquor, tissue, and biopsies. In particular the sample may be a blood sample or a gynaecological sample.

Because of these features the antibodies according to the invention are excellently suitable in the application of diagnostic problems, in which comparatively the tissue topological distribution analysis, e.g. determined by immunohistochemistry with quantitative expression parameters, e.g. obtained by Western Blot or immunoprecipitation, shall be analysed.

With the antibodies according to the invention the monospecific detection of the Mcm3 expression can be performed reliably in one-by-one conducted immunobiochemical detection methods such as ELISA, Western Blot, and immunoprecipitation, the Western Blot hereby being preferred, or immunohistochemical tissues, preferably on routine fixed and paraffin embedded tissue. For this the antibodies according to the invention may be labelled, if it is appropriate, as described above, or employed in combination with labelled antibodies directed against them or other reagents.

Monoclonal antibodies according to the invention can inhibit in vivo the assembly of DNA precursors and therefore inhibit cell proliferation. Thus, these

antibodies or the above mentioned derivatives of the same are suitable for the therapy of states of a disease which are accompanied by raised cell proliferation. Examples of such diseases are tumours, allergies, autoimmune diseases, scar formation, inflammations and rheumatic diseases as well as the suppression of defence reactions of transplantations.

For the production of pharmaceutical composition or a medicine the monoclonal antibodies according to the invention can be used alone or combined with common carriers, adjuvants and/or additives. The antibodies are suitable for the systemic, local, subcutaneous, intrathecal and topical application and for application by enema. For this they can be applied solved in suitable solvents, preferably as aqueous solution, in the form of liposomes, as emulsion or in solid state, e.g. as powder or in the form of microcapsules.

Alternatively, a monoclonal antibody of the present invention can be administered in a combined method of treatment with a different pharmaceutically active agent. Pharmaceutically active agents, that can be formulated with the monoclonal antibodies of the present invention, or alternatively can be administered in a combined method of treatment, can be for instance antibodies, in particular monoclonal antibodies, against other antigens, thus providing a "cocktail" containing a monoclonal antibody of the present invention and one or more (monoclonal) antibodies against other antigens involved in the pathogenesis of the relevant disease state.

Further active agents, that can be formulated with the monoclonal antibodies of the present invention, or alternatively can be administered in a combined method of treatment, especially in order to produce a

therapeutically useful effect, depend on the disease state to be cured and are, for instance, commercially available gamma globulin and immune globulin products, antibiotics, antimicrobial products, antibacterial and antitumor agents or a mixture of two or more of them.

Monoclonal antibodies according to the invention can be employed in a particularly advantageous way in the therapy of tumours, namely as such or in combination with other therapeuticals and forms of therapy respectively, such as radiatio, that is resistant against conventional tumour therapeuticals. Such resistances occur in unspecific cytostatica such as vinblastin or cisplatin either secondarily, i.e. after repeated application, or exist primarily at certain tumours, such as carcinoma of the kidneys.

The dosages of such antibodies will vary with the condition being treated and the recipient of the treatment, but will be in the range of 1 to about 100 mg for an adult patient preferably 1 to 10 mg usually administered daily for a certain period. A two part dosing regime may be preferable wherein 1 to 5 mg are administered.

In another preferred embodiment, the detection of Mcm3 is conducted in combination with the detection of the proteins Ki-67 and p27.

One of the most cited cell cycle associated proteins, used for histopathologic diagnostics within the past 16 years, is the Ki-67 protein (Scholzen T and Gerdes J (2000) J Cell Physiol 182: 311-322; Gerdes J, Schwab U, Lemke H and Stein H (1983). Int.J.Cancer 31, 13-20). The Ki-67 protein is expressed in proliferating cells, but rapidly disappears when cells enter a resting state

(Baisch, H, and Gerdes, J (1987). Cell Tissue Kinet. 20(4), 387-391).Clinical studies have shown that the Ki-67 antigen is an independent prognostic marker in many different human neoplasms, e.g. breast cancer (Jansen RL, Hupperets PS, Arends JW, Joosten-Achjanie SR, Volovics A, Schouten HC, and Hillen HF (1998). Br. J. Cancer, 78: 460-465), soft tissue sarcoma, meningeomas (Perry A, Stafford SL, Scheithauer BW, Suman VJ, and Lohse CM (1998). Cancer 82: 2262-2269), prostate cancer (Mashal RD, Lester S, Corless C, Richie JP, Chandra R, Propert KJ, and Dutta A (1996) Cancer Res 56(18):4159-63) and non-Hodgkin lymphoma (Gerdes et al. (1984), J. Immunol. 133: 1710-1715).

The protein p27 belongs to the family of cyclin dependent kinase inhibitors (CDKI), which regulate cell cycle progression by binding and inactivating cyclin-dependent-kinases complexes at defined checkpoints within the cell cycle (Toyoshima H, and Hunter T (1994) Cell 78(1):67-74). The expression of p27 serves as a robust marker for differentiation in normal developing tissue and also in tumors displaying deregulated growth (Lloyd RV, Jin L, Qian X, and Kulig E (1997) Am J Path 150: 401-407., Zhang P, Wong C, DePinho RA, Harper JW, and Elledge SJ (1998) Genes Dev 12(20):3162-3167).

Performing combined staining of tissues detecting the three proteins simultaneously, allow a more detailed assessment of cell proliferation and differentiation processes that determine individual tumor growth. MCM3 protein is expressed in cells that have ceased to proliferate, but are not terminally differentiated according to the absence of p27 protein expression, whereas Ki-67 is expressed in proliferating cells only. P27 can be found in quiescent cells but not in proliferating cells. Ki-67, MCM3 and p27 provide one set

of parameters which define complementary biological properties that are suitable for a detailed characterization of disordered cell growth and tumorgenesis. Tumor diagnostics may also benefit from a combined assessment of these markers which may be of help to choose the most appropriate therapy concept for an individual patient.

The present invention will be illustrated by the following examples:

EXAMPLES

Example 1

Production of monoclonal antibodies according to the invention.

Mice were used for immunisation. Recombinant human Mcm3 protein was used as antigen.

Record of immunisation and fusion
Day 1: 100 µg Mcm3 protein in 100 µl PBS (phosphate buffered saline) were mixed completely and thoroughly with 100 µl Freund's adjuvant and were injected subsequently into a mouse.

Day 14: 50 µg Mcm3 protein in 100 µl PBS were mixed completely and thoroughly with 100 µl Freund's adjuvant and were injected subsequently into a mouse.

Day 21: 50 µg Mcm3 protein in 100 µl PBS were mixed completely and thoroughly with 100 µl Freund's adjuvant and were injected subsequently into a mouse.

Day 37: 50 µg Mcm3 protein in 100 µl PBS were mixed completely and thoroughly with 100 µl Freund's adjuvant and were injected subsequently into a mouse.

On day 39 the mouse was killed painlessly. Spleen cells were removed and fused with myeloma cells. Hybridoma that reached full growth were obtained

Screening of hybridoma supernatant and cloning.

At first supernatants of the hybridoma which reached full growth were tested in a spot-blot-assay. For this 1 ml recombinant human Mcm3 in PBS (2 ng/ml) was placed on 1 cm x 0.5 cm sized pieces of nitrocellulose membrane. These pieces are placed in a 48 well-plate and dried for 15 minutes at room temperature. Subsequently incubation was made with blocking puffer (PBS, 0.005 Tween 20, 4% gelatine) for 45 minutes at room temperature. After several washing steps with PBS (0.05% Tween 20, 0.5% gelatine) incubation was made with the hybridoma supernatant for 60 minutes at room temperature. After several washing steps with PBS (0.05% Tween 20, 0.5% gelatine) a commercially available phosphatase-coupled goat-anti-mouse antibody, Dianova, Hamburg (dilution according to the instruction of the producer 1:10000) was added. After incubation for 1 hour at room temperature with PBS (0.05% Tween 20, 0.5% gelatine) the alkaline phosphatase detection reaction was conducted with the developer solution (36 mM 5' bromo-4-chloro-3-indolylphosphate; 400 mM nitro blue tetrazolium, 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) for 10 minutes at room temperature.

The hybridoma supernatant which were tested positive in the spot-blot were tested subsequently immunohistologically. For this paraffin sections, e.g.

tonsil, were dehydrated according to standard procedures (2 x 100% xylene, 2 x 100% EtOH, 2 x 70% EtOH, 2 x 40% EtOH), followed by washing briefly in water. The sections were then cooked in citrate buffer pH 6 (2.1g citric acid monohydrate for 1l, adjust with 2N NaOH to pH 6) in a pressure cooker for 1-5 minutes. After opening the cooker the sections were washed immediately in cold (RT) TBS, followed by incubation with hybridoma supernatant in a humid chamber for 30 minutes. After washing in TBS for several times antibodies bound to the sections were detected by means of the indirect immunoperoxidase method, stained with hemalum, embedded and evaluated microscopically.

The antibody according to the present invention shows the following staining pattern. The antibody predominantly reacts with the nuclei of cells in proliferative regions, indicated by the fact that cells of the dark zone within the germinal centers of human tonsils stain positive for Mcm3. Likewise cells near the basal layer of the normal mucosa react with the Mcm3 specific antibody. It should be noted that Mcm3 staining was also seen in the intermediate and upper layer which belong to the non-proliferating cell compartment of the oral mucosa.

Hybridoma which were positive both in the spot-blot and in immunohistology, were cloned and recloned until they were monoclonal. Independent monoclonal antibodies were obtained. A hybridoma cell line producing a monoclonal antibody according to the invention was deposited at the Deutschen Sammlung von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig under number DSM ACC2388 on February 16, 1999.

Example 2

Western Blot analysis of cell lysates with a polyclonal rabbit anti-Mcm3 antibody and a monoclonal antibody according to the invention.

Cell lysates of the cell line HELA (H) and CHO (C) were applied to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE). The proteins separated in the gel were transferred to a nitrocellulose membrane in a wet-blotting chamber over night. This membrane was then incubated with a diluted rabbit anti Mcm3 antiserum (Hu, B., et al., Nucleic Acid Res., 1993, 21: 5289-5293) (0.15 µg / ml) for 1 hour at room temperature. After several washing steps with PBS (0.05% Tween 20, 0.5% gelatin) a commercially available phosphatase-coupled goat-rabbit antibody (dilution according to the instructions of the producer, Dianova, Hamburg 1:10000)was added. After incubation of 1 hour at room temperature and once more washing in TNT-buffer (150 mM NaCl, 10 mM Tris ph 7.5, 0.05% Tween 20) the detection was conducted with the chemiluminescence method with the ECL system (Amersham Life Science, Braunschweig) according to the instructions of the producer.

As can be ascertained in Fig. 1, it turned out that the polyclonal anti-Mcm3 rabbit antibody revealed, besides the expected prominent main protein band with an apparent molecular weight of 105 kDa, further proteins in the molecular weight range between 50 kDa and 90 kDa.

The monoclonal anti-Mcm3 antibody according to the invention revealed only the expected protein band with an apparent molecular weight of 105 kDa.

Further, in immunohistochemical studies, the monoclonal antibody according to the present invention demonstrates its usefulness for the detection of Mcm3

Example 3
Immunoprecipitation with anti Mcm3 antibodies according to the invention

1 µg anti-Mcm3 primary antibody is added to 10 µl Dynabeads (Dynal M280 sheep-anti-mouse, Dynal, Hamburg) and incubated for 30 minutes at 4°C under rolling.

Cell preparations (1 x 10^6 Cells) are taken up in immunoprecipitation buffer (18 mM Tris/HCl, 150 mM NaCl, 0.3% hexadecylmethyl-ammoniumbromide, 5 mM EDTA and 1 mM DTT) comprising protease inhibitors, cooked for 5 minutes, cooled on ice and centrifuged (5 minutes, 14000 rpm). The excess liquid is added to the complex of Dynalbeads / primary antibodies and incubated at 4°C for 30 minutes on a roller.

Then the tube is placed in a Dynal magnetic concentrator for 20 seconds and the excess liquid is removed. Magnetic beads are resuspended in 500 µl NET (Tris/HCl 18 mM, NaCl 150 mM, EDTA 5 mM, DTT 1 mM) placed again in the magnetic concentrator and the supernatant is removed after 20 seconds. In this way, the beads are washed several times.

The so purified Mcm3 can then be analysed by means of SDS-PAGE.

Example 4
Micro injection of anti- Mcm3 antibodies according to the invention in nuclei of permanent cell line cells.

HEp-2 were cultivated on CELLocate® cover slips for microinjection and used in the logarithmic growth period. Anti-Mcm3 antibodies according to the invention and an irrelevant control antibody, respectively, were microinjected with a transjector and micro manipulator into the nuclei under light microscopy control (pressure of injection 130 hPa; time of injection between 0,3 and 0,5 seconds). The injected cells were then cultivated with bromodesoxyuridin (BrdU) containing (0.1 mM) medium for 6 hours. After fixation (5 minutes 4% paraformaldehyde at room temperature) the cover slips were washed three times in Tris-buffered saline (TBS), incubated in 100 % EtOH for 10 minutes at -20°C followed by permeation of the adhering cells by transferring directly in 0.1% Triton X-100 TBS for 10 minutes by room temperature. The injected antibodies were then detected with a commercially available Cy3 coupled goat anti mouse antibody, Dianova, Hamburg (dilution according to the instructions of the producer in PBS/10% bovine serum albumin). The preparations were first incubated in 2M HCl for 60 minutes at 37°C for the detection of BrdU fixed into the cells. Subsequently, the preparations were washed first several times with distilled water, then two times with PBS. Then incubation with a commercially available FITC labelled anti BrdU antibody, Boehringer Mannheim, Mannheim (dilution according to the instructions of the producer) overnight at 4°C in a humid chamber was performed. Then the preparations were washed thoroughly five times in PBS for 10 minutes, covered with DABCO (1,4-diazabicyclo[2,2,2]octane)in 90% glycerol, and evaluated by fluorescence microscopy.

It turned out that almost all cells injected with control antibodies incorporated also BrdU, hence passing during the experiment the normal cell cycle. In contrast only between 20% to 50% of the cells that had been injected

with anti-Mcm3 antibodies according to the invention incorporated BrdU. The proliferation of these cells was therefore inhibited by the antibodies according to the invention.

5

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CLAIMS

1. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically.

2. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically, whereby the monoclonal antibody has the same properties as the monoclonal antibody of the hybridoma cell line with the deposit number DSM ACC2388.

3. A monoclonal antibody detecting and binding monospecifically human Mcm3 both immunohistologically and immunobiochemically, whereby the epitope of the monoclonal antibody of the hybridoma cell line with the deposit number DSM ACC2388 is detected.

4.The monoclonal antibody according to claim 1, wherein the monoclonal antibody may be altered biochemically or molecular biologically or may be synthetic whereby the antibody lacks completely or partly portions that are necessary or unnecessary for the detection of Mcm3 or said portions are substituted by others imparting the antibody with further advantageous properties.

5. The monoclonal antibody according to claim 1, which is produced by the hybridoma cell line with the deposit number DSM ACC2388.

6. A hybridoma cell line which expresses a monoclonal antibody, detecting monospecifically and binding human Mcm3 both immunohistologically and immunobiochemically.

7. A hybridoma cell line according to claim 6, whereby the hybridoma cell line is the cell line with the deposit number DSM ACC2388.

8. Use of the monoclonal antibody according to any one of claims 1 to 5 in a detection method for human Mcm3.

9. Use of the monoclonal antibody according to any one of claims 1 to 5 for the immunohistological, immunocytological or immunobiochemical detection of human Mcm3 in a sample.

10. Use according to claim 9, characterised in that the sample is selected from a group consisting of serum, sputum, urine, and liquor.

11. Use according to claim 9, wherein the sample is tissue or fine needle aspiration.

12. Use according to claim 9, wherein the immunobiochemical detection comprises the methods ELISA, RIA, Western Blot, Far Western Blot, immunoprecipitation and affinity chromatographic steps.

13. Use according to claim 9, wherein the immunocytological method comprises FACS and MACS.

14. Use according to claim 9, wherein the immunohistochemical detection comprises fluorescence, radioactive, enzymatic and chemiluminescence methods.

15. A process for the production of the antibody according to any one of claims 1 to 5, characterised in that an animal is immunised with human Mcm3, and monoclonal antibodies are obtained after the fusion of

spleen cells of the animal with myeloma cells which comprises the steps :

(i) initial screening of the hybridoma by means of an immunobiochemical method

(ii) screening of the hybridoma that where positive in step (i) by means of an immunohistochemical method.

16. A process for the production of purified human Mcm3, characterised in that the monoclonal antibody according to any one of claims 1 to 5 is used.

17. A process for the production of purified human Mcm3, characterised in that the process comprises an affinity chromatography step with a monoclonal antibody according to any one of claims 1 to 5.

18. A process for the production of purified human Mcm3, characterised in that the process comprises an immunoprecipitation step with a monoclonal antibody according to any one of claims 1 to 5.

19. A diagnostic composition comprising a monoclonal antibody according to any one of claims 1 to 5.

20. Use of a monoclonal antibody according to any one of claims 1 to 5 for the production of a preparation for the therapy of tumours, allergies, auto-immunopathies, scar formation, inflammation and rheumatic diseases as well as the suppression of defense reactions of transplantations.

21. Pharmaceutical composition comprising monoclonal antibodies according to any one of claims 1 to 5 together with pharmaceutical acceptable adjuvants.

22. Diagnostic kit comprising the monoclonal antibody according to any of claims 1 to 5.

23. Diagnostic kit for the combined detection of the expression of Mcm3, Ki-67 and p27 for tumour diagnosis.

24. A method of preventing or treating a disease caused by or contributed by the activity or level of Mcm3 expression, comprising adminstering to the subject an effective amount of a pharmaceutical composition comprising an antibody according to any of claims 1 to 5, together with a pharmaceutically acceptable carrier.

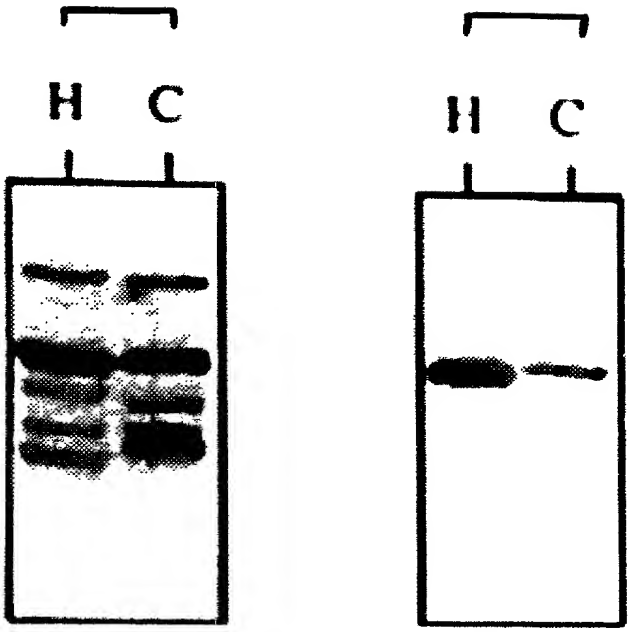

Fig.1

#3

DOCKET NO. 3276.1000-000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Declaration for Patent Application

As a named inventor, I hereby declare that:

My residence, mailing address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed) or an original, first and joint inventor (if plural names are listed in the signatory page(s) commencing at page 3 hereof) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST HUMAN PROTEIN MCM3, PROCESS FOR THEIR PRODUCTION, AND THEIR USE

the specification of which (check one)

[ ] is attached hereto.

[X] was filed on March 31, 2000 as PCT International Application No. PCT/EP00/02910, the U.S. National Phase of which was assigned U.S. Application No. 09/937,649, filed on September 27, 2001 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Not Claimed	Certified Copy Filed? YES	NO
DE 199 15 057.5 (Number)	Germany (Country)	01/April/1999 (Day/Month/Year filed)	[ ]	[ ]	[ ]
(Number)	(Country)	(Day/Month/Year filed)	[ ]	[ ]	[ ]
(Number)	(Country)	(Day/Month/Year filed)	[ ]	[ ]	[ ]

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information known by me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing date)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Filing date)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Filing date)	(Status: patented, pending, abandoned)
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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8-00

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9-00

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